29-46. (Canceled)

47. (Previously presented) The method of claim 1, wherein the RNaseIII enzyme is dicer.

48. (Currently amended) A method for attenuating expression of a target gene in mammalian cells, comprising introducing into mammalian cells suspended in culture an expression vector encoding a hairpin RNA which when transcribed from said expression vector in said ~~into the~~ mammalian cells ~~in an amount sufficient to~~ attenuates expression of the target gene, wherein the transcribed hairpin RNA:

(i) is a single nucleic acid strand having a double stranded portion including first nucleotide sequence of the target gene, and a second nucleotide sequence which is a complementary inverted repeat of said first nucleotide sequence and hybridizes to said first nucleotide sequence to form a hairpin structure;

(ii) is a substrate for cleavage by an RNaseIII enzyme to produce a double-stranded RNA product,

(iii) does not produce a general sequence-independent killing of the mammalian cells, and

(iv) reduces expression of said target gene in a manner dependent on the sequence of said double stranded portion of the hairpin RNA.

REMARKS

Claims 1 and 48 have been amended, and claims 2-8, 11 16-27 and 29-46 are canceled. After entry of this amendment, claims 1, 9,10, 12-15, 28 and 47-48 will be pending in this case. The above amendment is offered merely to expedite allowance of claims directed to products currently be developed and marketed by Applicants and their licensee. Applicants are not acquiescing to the pending rejections in the last office action; rather they reserve the right to prosecute claims of the same or similar scope in other applications.

The amended claims are fully supported by the specification. Support for the use of expression constructs to produce hairpin RNA for inducing gene silencing can be found, for example, at pages 28 and 43-44 and in Example 3 furthermore, Applicants note that the original claims 20-22, which were directed to use of hairpin RNAs , also provides support for the amended claims.

The Claims Are Patentable Over Li et al Reference

As set forth in the response filed November 2004, in considering the effect of the Li et al. Published Application, it is important to also understand that it was necessary to know the mechanism by which RNA interference ("RNAi") works in order to appreciate that expressed hairpin RNAs could be used to induce gene silencing by an RNA interference mechanism in mammalian cells suspended in culture. That is, in order for those skilled in the art to reasonably believe that a hairpin RNA could induce gene silencing, they first needed to understand the cellular mechanism by which double stranded RNA could induce sequence-specific gene silencing. As detailed in the Hannon Declaration submitted in November 2004, at the time of the filing of the Li et al. Published Application, that mechanism was not known to the public nor described in the Li et al. Published Application. The Li et al. Published Application is entirely silent on the mechanism of RNA interference, and contains only a single sentence referring to hairpin constructs – failing to provide an enabling disclosure of the claimed methods of the present application.

A. It was not obvious RNA interference would work in cultured cells.

At the time the Li et al. Published Application was filed in January 2000, procedures based on double stranded RNA-triggered silencing were fairly well-established tools for functional genomics of lower organisms (plants, invertebrates and fungi). The ability of a few molecules of double stranded RNA to eliminate a much larger pool of endogenous mRNA had suggested a catalytic or amplification component to the interference mechanism. For instance, some of the plant literature favored an RNA-based copying system that was proposed to produce copious amounts of antisense RNA (while perhaps also producing additional sense and dsRNA). See Jorgensen et al. (1998) Science 279: 1486; Waterhouse et al. (1998) Proc. Natl. Acad. Sci. 95:13959 and Wassenegger et al. (1998) Plant Mol. Biol. 37:349.

This experience, along with similar observations in *c. elegans* (see, e.g., Fire et al. (1998) Nature 391:806), suggested an amplification process in whole organisms. That is, that RNA interference was the consequence of a systemic response. *If that had indeed been the mechanism, it would not be apparent or expected that RNA interference using double stranded RNA would work on cultured cells, let alone that hairpin RNA could substitute for double stranded RNA in mammalian cells.*

B. It was not obvious that hairpin RNAs would work in mammalian cells

At the time the Li et al. Published Application was filed, one could certainly have hoped that RNA-triggered silencing would exist in vertebrates. However, the simple protocols used for invertebrate and plant systems were known to be uneffective in mammalian cells. At that time, it was recognized in the art that there were several impediments to the use of RNAi in normal mammalian cells. Most mammalian cells harbor a potent antiviral response that is triggered by the presence of dsRNA viral replication intermediates. Reviewed Williams (1997). Biochem. Soc. Trans. 25, 509-513 and Gil (2000). Apoptosis 5, 107-114. In somatic cells, dsRNA activates a variety of responses. Predominant among these is PKR, a kinase that is activated by dimerization in the presence of dsRNA (Clarke et al. (1995) RNA 1, 7-20). PKR, in turn, phosphorylates EIF2α, causing a nonspecific translational shutdown. dsRNA also activates 2′-5′ oligoadenylate polymerase, the product of which is an essential cofactor for a nonspecific ribonuclease, RNase L. Reviewed in Baglioni et al. (1983). Interferon 5, 23-42. The ultimate outcome of this set of responses is cell death via apoptosis.

The recapitulation of the essential features of RNAi was a prerequisite for a biochemical analysis of the phenomenon. In the absence of the biochemical and genetic approaches carried out by the inventors in several experimental systems and described in the instant application, those skilled in the art would have had no reasonable expectation that, based on the teachings of the Li et al. Published Application, hairpin RNA would have any effect as a gene silencing agent in mammalian cells. Understanding the mechanisms underlying RNAi in both invertebrates and vertebrates would have been required to recognize the utility of hairpin RNA for inducing gene silencing by an RNAi pathway. That understanding come from the work of the present inventors, who identified the existence of conserved machinery for double stranded RNA-induced gene silencing from drospohila to mammals. They also defined the RNA interference process as proceeding via a two-step mechanism. In the first step, double stranded RNA is

recognized by an RNase III family nuclease called Dicer, which cleaves the dsRNA into about 21-23-nt siRNAs (now called "small interfering RNA" or "siRNA"in the scientific literature). These siRNAs are incorporated into a multicomponent nuclease complex, RISC, which identifies substrates through their homology to siRNAs and targets these cognate mRNAs for destruction.

C. Antedating of Li et al. Published Application

The above arguments notwithstanding, the Li et al. Published Application is not prior art to the subject matter of the pending claims. The Declaration of Gregory J. Hannon under 37 CFR 1.131 submitted with the response filed November 2004 establishes that the inventors had possession of the subject matter disclosed in Li et al. Published Application, the effective date of that reference.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants submit that the pending claims are in condition for allowance. Early and favorable reconsideration is respectfully solicited. The Examiner may address any questions raised by this submission to the undersigned at 617-951-7000. Should an extension of time be required, Applicants hereby petition for same and request that the extension fee and any other fee required for timely consideration of this submission be charged to **Deposit Account No. 18-1945.**

Respectfully Submitted,

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Z. Angela Guo
Reg. No. 54,144

Customer No: 28120
Docketing Specialist
Ropes & Gray LLP
One International Place
Boston, MA 02110
Phone: 617-951-7739
Fax: 617-951-7050